FINAL REPORT

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CONTROL OF PARASITIC MITES IN HONEY BEES

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Control of Parasitic Mites in Honey Bees

Summary:

Laboratory Study Summary

A laboratory study was conducted to quantify the effects of different doses of formic acid on worker honey bees and varroa mites at four temperatures. Formic acid tended to be most effective in killing mites at higher temperatures. The survival curve data indicated that the best control of mites occurred when a high dose of formic acid was used for a short period of time. However, alternative methods also were shown to be possible. Significant differences in the killing rate of formic acid against bees and mites were found at higher doses of formic acid when temperatures where held at 15 and 35°C. Thus, it should be possible to fumigate using a dose of formic acid that would kill mites without harming bees.

A second component of the study was designed to apply the knowledge gained from the laboratory studies in applying formic acid to colonies in indoor wintering facilities. This study examined the use of formic acid as a fumigant to treat varroa infested colonies that are held in an indoor wintering facility. Colonies were kept under total darkness at a temperature of 5°C. Colonies were placed in four small rooms that were fumigated with different doses of formic acid for 48 hours. Formic acid concentration in room and hive air was monitored during fumigation. Queen, worker bee, varroa mite, tracheal mites and nosema levels within each colony were monitored at various times throughout the winter months.

This study showed that formic acid fumigation of bees within wintering buildings is feasible but there are several problems that still need to be solved before this can be carried out on a commercial scale. We have shown that a "high" formic acid concentration (45 ppm in room air for two days) is effective in reducing varroa infestations to levels below the economic threshold. The amount of chemical required to achieve effective control is much lower than in conventional treatments. However, these same dose-time combinations cause excessive queen loss (33%). Lower doses do kill some mites without killing queens, but single applications at these doses are not effective enough to reduce mite infestation levels below the threshold. We also showed that higher doses of formic acid also appear to suppress the bee dysentery disease *Nosema apis*. Levels of tracheal mites in honey bees exposed to different levels of formic acid were not affected in our experiment. In order to make this treatment method commercially viable we still need to establish the optimal dose-time combination and number of doses required to provide effective control without harming queens.

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Technical Report
Background and Objectives

The varroa mite is an external parasite of the honey bee. To date the only economical way of controlling this parasite is through the use of chemical acaricides. Fluvalinate strips have proven to be an effective and economical control measure for this parasite, however, it is important to have a number of chemical controls that have different physiological "modes of action" so that options will be available if mites develop resistance to one or more of these compounds. Resistance of mites to fluvalinate in Italy has been known since 1993 (Watkins, 1997; Bee World 78:15-22). Formic acid is the only other chemical that is currently registered in Canada for use in controlling the varroa mite in Canada and thus would be a logical alternative for use in a rotation schedule designed to delay the development of resistance to fluvalinate. Formic acid can be effective in controlling the mite, however, it is not a reliable option for all situations (Gatien and Currie 1994). Current control recommendations require up to five applications of formic applied in a "pour-on" formulation to achieve effective control of the varroa mite. This method of application is too labour intensive for many commercial beekeepers in the prairie regions of Canada where the required number of treatments can not be applied in the short period available prior to and following the honey flow.

Different formulations of formic acid may be more effective from the standpoint of both reducing labour inputs and increasing efficacy. For example, a slow-release formulation would be beneficial for spring, fall and possibly winter application of formic acid. Initial trials with a slow-release method of applying formic have indicated that effective control of the mite can be achieved Currie and Gatien 1995). Unfortunately, in the prairie region, spring applications with a slow release of formic acid appear to reduce honey production (Gatien and Currie, 1995 unpublished data). This reduction in honey production may be caused by a disruption of brood rearing in colonies are continually exposed to formic acid during the critical phase of honey bee population growth in the spring.

Another alternative method of formic acid application would be a winter treatment. This could be feasible in the prairie provinces of Canada where many beekeepers winter their colonies indoors. Outdoor formic acid applications involve treating individual colonies under highly variable environmental conditions. The fumigation of entire buildings could allow treatment of hundreds of colonies at the same time under highly controlled conditions.

This research will determine the precise conditions under which formic acid is effective and under which it may have a negative impact on colony performance. Formic acid will be a crucial alternative to fluvalinate strips for controlling of varroa mite when the mites develop resistance to the later compound.

The objectives of this research are (1) to determine the optimal doses of formic acid that are required to control the varroa mite under the wide range of environmental conditions experienced in the prairie region; (2) to determine lethal and sublethal doses of formic acid for workers and queens under a variety of environmental conditions; (3) to assess the efficacy of different release rates, timing of application and method of application to control the varroa mite; and (4) to assess

the effect of different release rates, timing of application and method of application on colony performance and honey production.

Project Activities:achievments

The results of this project will produce significant economic benefit to honey producers throughout the prairie provinces. Over 400,000 colonies of honey bees are wintered each year across the prairies, of which approximately half are wintered indoors. The current costs of varroa control are estimated at $4 per hive (using fluvalinate strips), not including labour. Development of the technology outlined in this project could reduce the costs of treatment per colony to approximately 15 cents per hive and also substantially reduce labour costs. The economic savings in chemical costs from this one chemical in one year would total approximately $770,000 from direct reductions in the chemical costs alone. Beekeepers would receive even greater benefit from the reduced labour costs associated with numerous treatment applications that are required when formic acid is applied to colonies in the spring and in the fall. Beekeepers also implement separate control programs for tracheal mite and *Nosema apis* and savings in input costs associated with these control measure might also be obtained through winter-fumigation.

The results of this research have been widely distributed through oral presentations at a variety of beekeeper meetings. Two publications have been produced. One on the laboratory component of the study and another on the field testing of compounds in the overwintering building. These publications with be submitted shortly for publication in refereed scientific journals. Additional achievements related to this project that are associated with the application of formic acid in individual colonies are contained in the report on Saskatchewan Agrifood Innovation project 970000001.

Papers:

Underwood, R.M and R.W. Currie. 2001. Indoor winter fumigation of Apis mellifera L (Hymenoptera: Apidae) colonies infested with Varroa destructor Anderson and Truman (Acari: Varroidae) is a practical control alternative in northern climates.

Underwood, R.M and R.W. Currie. 2001. The effects of temperature and dose of formic acid on efficacy of treatment of Varroa-infested honey bees.

Presentations

(3) Abstracts, Papers in Conference Proceedings:

Underwood, R.M. (2001) Formic acid fumigation of overwintering honey bees infested with *Varroa jacobsoni*. Entomological Society of Canada-Entomological Society of Ontario. Joint Annual Meeting. Niagra Falls, Ontario

Underwood, R.M. (2000) Formic acid fumigation of overwintering honey bees infested with *Varroa jacobsoni*. *Prairie Universities Biological Symposium PUBS 2000, Regina, SK, February*. Pp 29-30

Underwood, R. and Currie, R. W. (2000) Mighty bee-mite might be doomed. Winter fumigation of varroa-infested honey bee colonies is a practical control alternative. Control of *Proceedings, Entomological Society of Manitoba '56th Annual Meeting. Winnipeg, 5-6 November* (Abstract *in press*: Paper Presented by RU).

Underwood, R. and Currie, R. W. (1999) Effect of temperature and dose of formic acid on efficiency of control of *Varroa jacobsoni*. *Proceedings, Apimondia '99: XXXVI International Apicultural Congress*, p. 257, Sept. 1999, Vancouver, BC. (Poster presentation, Abstract).

Underwood, R. and Currie, R. W. (1999) Effect of temperature and dose of formic acid on efficiency of control of *Varroa jacobsoni*. *Proceedings, Entomological Society of Manitoba '55th Annual Meeting. Winnipeg, 5-6 November* (Abstract *in press*: Paper Presented by RU).

Underwood, R. 2001. Treating varroa with formic acid in indoor wintering facilities. Manitoba Beekeepers Association 91th Annual Meeting. Winnipeg, Manitoba. February.

Currie, R.W. 2001. Use of Formic Acid to Control Varroa Mites in the Wintering Building. Annual Meeting of the Saskatchewan Beekeepers Association. Saskatoon, Saskatchewan. February.

Currie, R.W. 2001. Economic Impact of Varroa Mites: Managing with Formic Acid. Annual Meeting of the Saskatchewan Beekeepers Association. Saskatoon, Saskatchewan. February.

Currie, R.W. 2000. Use of Formic Acid to Control Varroa Mites in the Wintering Building. Annual Meeting of the Alberta Beekeepers Association. Edmonton, Alberta. November, 2000.

Currie, R.W. 2000. Economic Impact of Varroa Mites: Managing with Formic Acid. Annual Meeting of the Alberta Beekeepers Association. Edmonton, Alberta. November, 2000.

Underwood, R. M. 2000 Formic acid fumigation of overwintering honey bees infested with *Varroa jacobsoni*. Manitoba Beekeepers Association 90th Annual Meeting. Brandon, Manitoba. February, 2000.

Currie, R.W. 2000. Varroa Mites: Managing with Formic Acid? Canadian Honey Council Research Symposium. February, 2000. Saskatoon, Saskatchewan

Underwood, R. M. 2000 Formic acid fumigation of overwintering honey bees infested with *Varroa jacobsoni*. Prairie Universities Biological Symposium, Regina, Saskatchewan, Canada, February 2000

Underwood, R. M. 2000 Formic acid fumigation of overwintering honey bees infested with *Varroa jacobsoni*. Red River Apiarists Association Meeting. Winnipeg, Manitoba. February, 2000.

Currie, R.W., 1999. Varroa mites: Are we managing with formic acid? Manitoba Beekeepers Association: 89th Annual Meeting. 9 February, 1999.

Currie, R.W. 1999. Honey bee research at the U. of M. Red River Apiarists Association. Winnipeg, Manitoba. 8 March, 1999.

Currie, R.W. 1999. Manitoba Beekeepers Association: 93rd Annual Meeting. Winnipeg, Manitoba. 6 February, 1996.

Results and Conclusions

This study was divided into three major components. First, was laboratory study to quantify the effects of different doses of formic acid on worker honey bees and varroa mites at four temperatures. The second component was designed to apply the knowledge gained from the laboratory studies in applying formic acid to colonies in indoor wintering facilities and the third component was designed to examine how different formulations of formic acid act on colony development, mites and other honey bee diseases when applied to colonies in the spring. The latter component of this research was carried out in conjunction with the Saskatchewan Agri-food Innovation Fund "Chalkbrood and Disease Control in Honey Bees" and the results of that research are contained in the report on project 97000001.

The details of the experiments associated with the laboratory study and the field testing of formic acid fumigation in the wintering building are described in the drafts of two scientific papers that are attached to this report.

Laboratory Study

The major conclusions of the laboratory study are summarized below:

1. Fumigation against varroa mites using formic acid is most consistently effective at doses greater than 0.08 mg/L.

2. Doses of .08 and .16mg/L were effective at reducing mite survival at all temperatures tested above 5°C and the highest dose (0.16mg/L) showed some effectiveness against varroa mites at 5°C .

3. Efficacy of a given dose of formic acid against varroa mites varies with temperature of fumigation.

4. The widest range of doses that were effective in reducing mite survival were found when fumigation was carried out at 25°C. However, concentrations above 0.08 mg/L were also effective at killing mites at 15 and 35°C..

5. Doses of formic acid that were most harmful to mites also harmed bees in our laboratory bioassay. Direct comparisons of the impact on bees relative to mites were carried out by multiplying the Formic acid concentration X the exposure time for both bees and mites. The result, the CT product, was then compared to determine the temperature dose combinations that provided the widest separation between bees and mites. The most dramatic differences between the CT products of mites and bees occurred at temperatures of 35°C at doses of 0.08 0.16 mg/L. However, there was also a significant difference between the CT product of mites and bees at 15°C when the dose was 0.08 mg/L.

Laboratory Study Summary

Formic acid tended to be most effective in killing mites at higher temperatures. The survival curve data indicated that the best control of mites occurred when a high dose of formic acid was used for a short period of time. However, alternative methods also were shown to be possible. Significant differences in the killing rate of formic acid against bees and mites were found at higher doses of formic acid when temperatures where held at 15 and 35°C. Thus, it should be possible to fumigate using a dose of formic acid that would kill mites without harming bees.

Wintering Building Fumigation Study

The major conclusions of the wintering building fumigation study are summarized below:

1. This study demonstrated that formic acid fumigation of colonies held within wintering buildings is a feasible method that could be used by commercial beekeepers to control varroa mites.

2. The method uses substantially less formic acid on a per colony basis than conventional treatments and thus would reduce chemical input costs.

3. The method uses substantially less labour on a per colony basis than conventional treatments and thus would also reduce labour input costs.

5. The dose of formic acid released into building air can be controlled through relatively simple methods.

6. The formic acid is evenly distributed throughout the room using the type of fan-jet ventilation that is commonly found in many commercial wintering buildings.

7. The formic acid in building air is much higher than in the hives themselves. The exposure dose calculations should be based upon the concentration of formic acid in hive air and not building air.

8. Some doses appear to reduce the levels of a common bee dysentery disease, *Nosema apis*. Many beekeepers routinely treat their colonies in the fall to control this disease with a prophylactic drug (fumagillin). If formic acid fumigation is found to provide reliable control of *Nosema* then producers could eliminate the input costs associated with prophylactic treatments for this disease.

9. None of the formic acid dose-exposure time combinations that we tested reduced the proportion of bees infested with tracheal mite relative to untreated control colonies. However, we did not measure tracheal mite mortality in our experiment. Some of the tracheal mites may have been dead but still present in the trachea.

9. The doses of formic acid that are most effective in controlling mites and *Nosema apis* also

cause substantial queen loss (~30%). Further development of this method will have to focus on finding ways of fumigating colonies to reduce mites without causing damage to queens.

Wintering Building Fumigation Study Summary

This study examined the use of formic acid as a fumigant to treat varroa infested colonies that are held in an indoor wintering facility. Colonies were kept under total darkness at a temperature of 5°C. Colonies were placed in four small rooms that were fumigated with different doses of formic acid for 48 hours. Formic acid concentration in room and hive air was monitored during fumigation. Queen, worker bee, varroa mite, tracheal mites and nosema levels within each colony were monitored at various times throughout the winter months.

This study showed that formic acid fumigation of bees within wintering buildings is feasible but there are several problems that still need to be solved before this can be carried out on a commercial scale. We have shown that a "high" formic acid concentration (45 ppm in room air for two days) is effective in reducing varroa infestations to levels below the economic threshold. The amount of chemical required to achieve effective control is much lower than in conventional treatments. However, these same dose-time combinations cause excessive queen loss (33%). Lower doses do kill some mites without killing queens, but single applications at these doses are not effective enough to reduce mite infestation levels below the threshold. We also showed that higher doses of formic acid also appear to suppress the bee dysentery disease *Nosema apis*. Levels of tracheal mites in honey bees exposed to different levels of formic acid were not affected in our experiment. In order to make this treatment method commercially viable we still need to establish the optimal dose-time combination and number of doses required to provide effective control without harming queens.

Indications for Further Development

Our research showed that formic acid slow-release formulations can have a negative impact on colony growth and development when applied to colonies in a spring under conditions found in the Canadian prairie region (see project number 970000001). There is a need for developing an improved form of formic acid release devise for use in the Canadian prairie region that will control mites without hampering colony growth. Our results from laboratory assays suggest that such a device should release spikes of formic acid over an extended period rather than releasing a slow consistent dose. This type of release rate would be more likely to increase rates of mite mortality without harming bees.

Our trials on releasing formic acid inside wintering buildings showed that varroa mites can be effectively and economically treated during the winter months. Some doses also appeared to provide control of a form of bee dysentery, *Nosema apis*. However, the doses of formic acid that were most effective in controlling both *Varroa* and *Nosema* our study caused substantial queen loss. More research is required to improve this technique to provide producers with method of treatment that will allow good mite control without harming queens. Such research will have to

determine the precise dose an exposure conditions needed to control varroa without harming queens.

Personnel

The following personnel were involved in this project. Robyn Underwood, M.Sc. Student, David Osterman (see project 970000001), M.Sc. Student, Linda Klymochko, technician, Nicole Froese, summer research assistant, Cristine Robinson, Summer research assistant.

Equipment

No major equipment was purchased for this project.

Innovation Fund Acknowledgment

The Saskatchewan Agri-Food Innovation Fund has been acknowledged in all oral and written presentations made on this research. It will also be acknowledged in all resulting scientific publications.

The effects of temperature and dose of formic acid on efficacy of treatment of *Varroa*-infested honey bees

Robyn M. Underwood and Robert W. Currie

Department of Entomology

University of Manitoba

Winnipeg, Manitoba, Canada R3T 2N2

Introduction

Varroa destructor Anderson and Trueman 2000 (Anderson and Trueman, 2000) is an introduced ectoparasitic mite that feeds on the hemolymph of adult and developing honey bees (*Apis mellifera* L.). This pest has caused millions of dollars in damages to the beekeeping industry due to loss of honey yield and destruction of honey bee colonies. Effective control methods are necessary to prevent severe losses.

The contact poison, fluvalinate-tau, is generally a highly effective control for this pest. However, resistance to this method of control is already a problem (e.g. Eischen, 1995, Harbo and Hoopingarner, 1997, Elzen *et al.*, 1998). Formic acid fumigation is an alternative control that has the advantages that no documented resistance to the chemical has occurred (VonPosern, 1988), it is inexpensive (Liu, 1991), does not leave residues in wax or honey (Stoya *et al.*, 1986, VonPosern, 1988, Liu *et al.*, 1993, Bogdanov *et al.*, 1999), and does not usually harm adult worker honey bees (e. g. Koeniger and Rau, 1980, Garg *et al.*, 1984, Lupo and Gerling, 1990, Westcott and Winston, 1999). However, the efficacy of this treatment method varies considerably. Even identical treatments have shown highly variable results (Charriere *et al.*, 1992).

There are a number of factors known to affect treatment efficacy. The distance from the site of formic acid volatilization to the comb and the placement of the acid in the hive above or below the brood chamber both affect treatment efficacy (Fries, 1989). Factors, such as the brood area within the hive (Eischen, 1998), time of year (Maul *et al.*, 1980), and ambient temperature (e.g. VonPosern, 1988, Feldlaufer *et al.*, 1997, Eischen, 1998, Calderone and Nasr, 1999) can all influence treatment efficacy.

The actual concentration of formic acid in the air in the honey bee hive during treatment varies with temperature and dosage. Unfortunately, researchers rarely take measurements of the actual dosage to which the honey bees are exposed (e. g. Feldlaufer *et al.*, 1997, Charriere *et al.*, 1992). Thus, variations in formic acid concentration in air, even with the same dose applied, could explain some of the variability in efficacy of treatments during tests. Measurements of the actual concentration of formic acid in the air in the hive are crucial for determining a standard for treatment with formic acid.

In order to improve the reliability and efficacy of treatment, we need to first determine the combination of dose and exposure time necessary to control mites. Formic acid treatments are generally reported as weights or volumes of acid evaporated into a hive for a given number of days. While these treatments may have been effective for those reporting them, they do not take into account environmental factors, such as ambient temperature, which may influence the actual concentration or amount of time the mites are exposed.

A more reliable value would be a concentration x time (CT) product. This value denotes the amount of acid acting on the mites over a given time period. This value is determined by experimentally assessing the amount of fumigant and length of time necessary to kill 50% of the test individuals (Bond, 1984). Various doses of the fumigant must be tested under a range of temperatures and for a period of time long enough to achieve significant control. A CT product is given as the concentration (measured in mg/l in air) multiplied by the amount of time it took to kill 50% of the test individuals at a given temperature. Theoretically, this value is constant over the range of doses and times (i.e. a short, high dose treatment should cause mortality equal to a long, low dose

treatment). The value is recalculated at each temperature. These values can then be used to extrapolate the amount of time and dose necessary to control a pest under practical conditions. Fumigation systems for controlling mites should be designed to ensure that the CT product is realized. Once the CT product is determined, recommendations for practical use of the fumigant can be given.

In this study, we assessed the efficacy of formic acid in a controlled laboratory environment under a range of temperatures and formic acid concentrations. A range of temperatures was chosen to cover the gradient of temperatures found within a cluster of honey bees. Doses were chosen within a range that was found to not cause immediate honey bee mortality in preliminary studies. The objectives of this research were 1) to determine the effect of temperature and dose of formic acid on worker honey bee and adult, female *Varroa destructor* survival and 2) to determine the concentration x time product for both *Varroa* mites and honey bees at a range of temperatures.

Methods and Materials

Experimental Setup. Bioassay cages had outer dimensions of 20.5 x 8.0 x 11.2 cm. Within each cage, bees were confined to a 15.8 x 8.8 x 5.0 cm space. The two sides, back and the top of the cage were solid wood, the front was a fine mesh screen and the floor was removable five-squares-per-inch hardware cloth. Below the floor was a drawer that could be removed for counting fallen mites and bees without allowing bees to escape. The cage top had two 3.5 cm diameter holes into which 50 ml centrifuge tube feeders were placed. The lid of each centrifuge tube had a 2 cm hole cut into it that was

covered with a fine mesh. One feeder tube contained distilled water and the other contained 67% sucrose solution. Each tube was refilled when necessary.

Mixed age worker honey bees were collected from colonies with no or low mite loads, anesthetized with CO_2, counted, and placed in the cages in groups of 300. Each cage was than infested with 30 live *Varroa* mites. Mites were collected by a water wash as follows. Frames of bees from highly infested colonies were anesthetized with CO_2 and shaken into a margarine container with a hardware cloth (five-squares-per-inch) lid. The container was then placed in a bucket of warm, distilled water and allowed to soak for five minutes. After soaking, the containers were shaken in the water vigorously to dislodge mites. The water in the bucket was then poured through a fine mesh cloth that trapped the mites. The mites were then picked up with the tip of a soft paint brush and placed on a paper towel. Mites quickly recovered and were introduced into bioassay cages. The bees were placed on a paper towel on top of the frames of the hive where they recovered.

The cages were then placed in temperature-controlled chambers and held under constant darkness at 30°C for 24 hours. On day 0, cages were randomly assigned to each treatment combination. Bioassay cages were placed in a flow-through fumigation system **(Fig. 1)** for the duration of treatment; up to day 12. Humidified air was blown through a clear vinyl tube (0.63 cm inner diameter) at a rate of 400 ml/min into a 1L mason jar containing 300 ml of a known concentration of formic acid in distilled water (Table 1). The air was bubbled through the liquid and pushed through a tube that led to the bioassay cage. The cage was confined in a closed Mylar bag (LOOK, city) to prevent leakage of any formic acid vapor into the chamber. Air coming from the bag was exhausted through

an additional tube into a fume hood. Bioassay cages were treated with six doses of formic acid; 0.00, 0.01, 0.02, 0.04, 0.08, and 0.16 mg/l (0, 5, 10, 20, 40 and 80 parts per million). Each dose was tested at each of four temperatures; 5, 15, 25, and 35°C. Thus, there was a total of 24 treatments and the experiment was replicated three times. Within each replicate, all of the worker bees were taken from a single colony and a different colony was used for each replicate. *Varroa* mites were from a number of colonies and assigned to each cage without knowledge of their origin. Fumigation was continuous throughout the treatment period.

Each day, dead bees and mites were removed from the test cages and counted. Fumigation ended after 12 days and any remaining live bees and mites were anesthetized with CO_2 and counted.

Formic Acid Concentration. Prior to the onset of the experiment, the concentration of formic acid in the air within the system was measured using an air sampling system (National Dräger, Pittsburgh, PA). A gas detector pump (Accuro 9022003) was used to suck air through a Dräger tube (no. 6722101) as per the manufacturer's instructions. Graduations (in parts per million) on the side of the tube allowed for an immediate reading of the formic acid concentration in the air represented by a color change in the indicator material. A standard curve for each temperature was created to determine the percent of acid in the jar that gave a desired formic acid concentration in air. Once the standard curve was created, acid concentration in air was no longer monitored.

Data Analysis. Mite and worker bee survival at each dose of formic acid was compared to survival of the control within each temperature treatment using the

LIFETEST procedure in SAS (SAS Institute Inc. 1999). The CT product for each cage was calculated to determine the combination of dose and time of exposure that caused 50% mortality in mites and worker bees after correcting for mortality in the control using Abbott's formula {Abbott 1925 #64}. The CT product data were analyzed using a split-plot design with formic acid dose, temperature, and replicate as main plot factors and species within cage as a subplot factor. Comparisons of the CT products between bees and mites within each temperature and dose and the effects of dose and temperature on the CT product of each species were analyzed using orthogonal contrasts with Bonferroni-corrected alpha values.

Results

Survival. Mite survival was affected by formic acid dose, but the survival response to formic acid varied with temperature. At 5°C formic acid treatment reduced mite survival only at a dose of 0.16 mg/l and only at early survival times as indicated by the Wilcoxon test (Figure 2d). The two highest doses were effective in killing mites at all other temperatures (Figure 2a-c). However, at 25°C, all doses tested (0.01 mg/l or higher) reduced mite survival relative to controls (Figure 2b).

At all temperatures, honey bee survival relative to controls was impacted by formic acid treatment (Figure 3). However, the effect of formic acid was not always negative. Bee survival was slightly higher than in untreated controls at doses of 0.02 and 0.04 mg/l at 5°C (Figure 3d). Bees showed reduced survival relative to controls for most formic acid doses at temperatures higher than 5°C (Figure 3a-c). At 15°C, treatments of

0.04 mg/l or-higher reduced bee survival (Figure 3c) and at 25 and 35°C, all doses reduced bee survival relative to controls (Figure 3a-b).

CT Products. The CT products of bees and mites at various temperature and dose combinations are shown in Figure 4. There was a significant interaction between temperature, dose, and species ($F=4.25$; $df=12, 40$; $P=0.0003$) for the CT product required to kill 50% of the individuals in each treatment. At 35°C there was a significant difference between the CT products of bees and mites for the doses of 0.08 mg/l and 0.16 mg/l (0.08 mg/l: $F=23.56$, $df=1$, $P<0.025$; 0.16 mg/l: $F=94.24$, $df=1$, $P<0.025$). At 15°C the difference was significant in the CT product of bees and mites only for a dose of 0.08 mg/l ($F=16.36$; $df=1, 40$; $P<0.05$). There were no differences between the CT products of bees and mites at 5 and 25°C or at doses of 0.04 mg/l or lower at 15 and 35°C.

The CT product of both bees and mites was significantly affected by temperature at formic acid concentrations of 0.04, 0.08, and 0.16 mg/l (bees, 0.04 mg/l: $F=10.80$, $df=3, 40$; $P<0.025$; bees, 0.08 mg/l: $F=65.00$; $df=3, 40$; $P<0.025$; bees, 0.16 mg/l: $F=32.58$; $df=1,40$; $P<0.025$; mites, 0.04 mg/l: $F=17.56$, $df=3, 40$; $P<0.025$; mites, 0.08 mg/l: $F=51.06$; $df=3, 40$; $P<0.025$; mites, 0.16 mg/l: $F=155.23$; $df=3, 40$; $P<0.025$) (Figure 3). The CT product changed with temperature, but the response differed for each dose. For example, for both bees and mites at the dose of 0.16 mg/l, the CT products decreased as temperature increased. On the other hand, at a dose of 0.08 mg/l, the CT products increased between 5 and 15°C, decreased between 15 and 25°C, and increased again between 25 and 35°C.

Formic acid concentration also significantly affected the CT product of bees and mites at all temperatures (bees, 5°C: $F=128.50$; $df=4, 40$; $P<0.025$; bees, 15°C: $F=110.04$;

df=4, 40; P<0.025; bees, 25°C: F=67.04; df=4, 40; P<0.025; bees, 35°C: F=36.79; df=4, 40; P<0.025; mites, 5°C: F=148.01; df=4, 40; P<0.025; mites, 15°C: F=74.15; df=4, 40; P<0.025; mites, 25°C: F=62.57; df=4, 40; P<0.025; mites, 35°C: F=10.05; df=4, 40; P<0.025). For both bees and mites at 15 and 35°C, the CT product increased from 0.01 to 0.08 mg/l and decreased from 0.08 to 0.16 mg/l. In contrast, at 5 and 15°C, the CT product increased or stayed the same from 0.01 to 0.16 mg/l.

Discussion

The efficacy of fumigation of *Varroa*- infested honey bees with formic acid is variable, even under controlled conditions. The temperature during fumigation affects efficacy even with the same concentration of formic acid being applied. Only a few temperature-dose combinations were more effective at killing mites than bees.

The two highest doses tested, 0.08 and 0.16 mg/l, were effective at reducing mite survival at temperatures above 5°C. Therefore, we do not recommend fumigation with doses less than 0.08 mg/l or when bees are at 5°C.

Because of the social nature of honey bees, treatment temperature is not necessarily the temperature to which the insects are exposed. Honey bees cluster together at low temperatures, creating a higher than ambient temperature in the cluster which is necessary for survival. In a treatment at 5°C, a cluster of more than 300 bees is required. During this study, 200 or more bees died on a single day on several occasions because they were chilled, not because of the acid treatment. The mites on these bees were alive, but were considered dead the next day due to a lack of hosts. Therefore, the efficacy of formic acid fumigation at 5°C was not able to be properly assessed in this study.

Fumigation at this low temperature will only be possible if a larger cluster of bees is treated, as in a colony.

Honey bee survival was compromised in all treatments, including the controls. In a colony, bees are free to move throughout the gradient of temperatures. In the laboratory, test animals were forced to remain at one temperature for a long period of time. This may be the cause of some of the mortality. This does not take away from the results here, though, since mortality in the controls was accounted for with the use of the Abbott's formula correction {Abbott 1925 #64}.

In this laboratory study, honey bee survival was reduced by formic acid fumigation. However, at 15 and 35°C, the difference between the CT products of bees and mites differed significantly at some acid concentrations. There is, therefore, a time period when mites can be killed without increasing bee mortality. Future studies should investigate the use of short-term, high dose fumigation possibilities in addition to the popular long-term, low dose fumigation.

Temperature of fumigation had a significant impact on the CT products of both bees and mites. There was not a general trend over all doses, indicating that the effect of temperature on fumigation efficacy may be unpredictable. This may, however, be due to the fumigation method employed in this study. Formic acid concentrations were only measured in a pre-trial assay. Therefore, changes in acid concentration in air over time were not detected. Monitoring the acid concentration is expensive, but will be necessary to assure consistent acid levels throughout the treatment period.

In addition to temperature, the dose of formic acid affected the CT product. This may be due to the fact that the low doses, 0.01, 0.02 and 0.04 mg/l, were beyond the

stable portion of the concentration x time curve. Future researchers may consider eliminating these treatments altogether.

The CT product is a reliable value that can be used to standardize treatments with fumigants. This study is the first to determine a CT product for formic acid fumigation of *Varroa*-infested honey bees. Because the CT product changes with dose and temperature, close monitoring of treatments is necessary when using formic acid. More research is necessary to elucidate the cause of efficacy variability and to standardize the use of this mite control method.

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Literature Cited

Anderson, DL and Trueman, JWH, 2000. *Varroa jacobsoni* (Acari: Varroidae) is more than one species. Experimental and Applied Acarology 24:165-189.

Bogdanov, S., Kilchenmann, V., Fluri, P., Bühler, U., and Lavanchy, P., 1999. Influence of organic acids and components of essential oils on honey taste. American Bee Journal 139:61-63.

Bond, EJ, 1984. Manual of Fumigation for Insect Control. Research Centre, Agriculture Canada, London, ON.

Calderone, N. W. and Nasr, M. E., 1999. Evaluation of formic acid formulation for the fall control of *Varroa jacobsoni* (Acari: Varroidae) in colonies of the honey bee *Apis mellifera* (Hymenoptera: Apidae) in a temperate climate. Journal of Economic Entomology 92:526-533.

Charriere, J. D., Imdorf, A., and Kilchenmann, V., 1992. Formic acid concentration in the air in a beehive. Schweizerische Bienen-Zeitung 115:463-469.

Eischen, F., 1995. Varroa resistance to fluvalinate. American Bee Journal 135:815-816.

Eischen, F. A., 1998. Trials (and tribulations) with formic acid for Varroa control. American Bee Journal 138:734-735.

Elzen, P. J., Eischen, F. A., Baxter, J. B., Pettis, J., Elzen, G. W., and Wilson, W. T., 1998. Fluvalinate resistance in *Varroa jacobsoni* from several geographic locations. American Bee Journal 138:674-676.

Feldlaufer, M. F., Pettis, J. S., Kochansky, J. P., and Shimanuki, H., 1997. A gel formulation of formic acid for the control of parasitic mites of honey bees. American Bee Journal 137:661-663.

Fries, I., 1989. Short-interval treatments with formic acid for control of *Varroa jacobsoni* in honey bee (*Apis mellifera*) colonies in cold climates. Swedish Journal of Agricultural Research 19:213-216.

Garg, R., Sharma, O. P., and Dogra, G. S., 1984. Formic acid: an effective acaricide against *Tropilaelaps clareae* Delfinado and Baker (Laelaptidae: Acarina) and its effect on the brood and longevity of honey bees. American Bee Journal 124:736-738.

Harbo, J. R. and Hoopingarner, R. A., 1997. Honey bees (Hymenoptera: Apidae) in the United States that express resistance to *Varroa jacobsoni* (Mesostigmata: Varroidae). Journal of Economic Entomology 90:893-898.

Koeniger, N. and Rau, C., 1980. Field trials in 1979/1980 with formic acid in

Hochtaunuskreis. Allgemeine Deutche Imkerzeitung 14:157-159.

Liu, T. P., 1991. Formic acid and bee mites. American Bee Journal 131:311-312.

Liu, T. P., Chu, L. T. Y., and Sporns, P., 1993. Formic acid residues in honey in relation to application rate and timing of formic acid for control of tracheal mites, *Acarapis woodi* (Rennie). American Bee Journal 133:719-721.

Lupo, A. and Gerling, D., 1990. A comparison between the efficiency of summer treatments using formic acid and Taktic® against *Varroa jacobsoni* in beehives. Apidologie 21:261-267.

Maul, V., Petersen, N., and Wissen, W., 1980. Field trials using formic acid as a varroatosis therapy. Allgemeine Deutsche Imkerzeitung 14:155-157.

Stoya, W., Wachendörfer, G., Kary, I., Siebentritt, P., and Kaiser, E., 1986. Formic acid as a therapy against Varroatosis and its absorption into honey. Deutsche Lebensmittel-Rundschau 82:217-221.

VonPosern, H., 1988. Stopping Varroa's victory march part II. American Bee Journal 128:425-428.

Westcott, LC and Winston, ML, 1999. Chemical acaricides in *Apis mellifera* (Hymenoptera: Apidae) colonies; do they cause nonlethal effects? Canadian Entomologist 131:363-371.

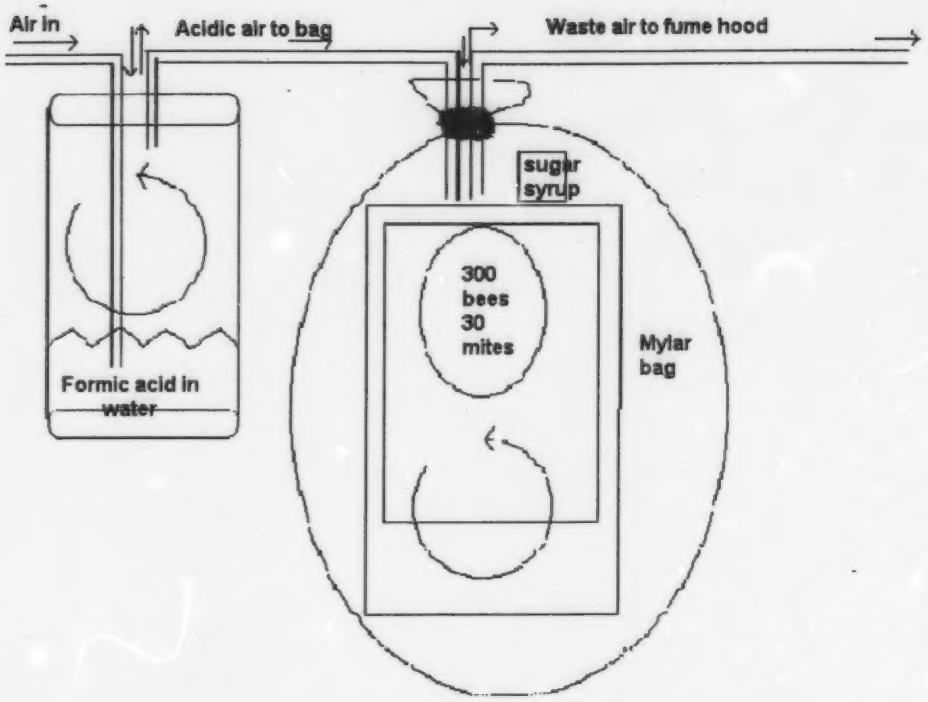

Figure 1. Laboratory setup. Humidified air was forced through a vinyl tube into a Mason jar containing a given concentration of formic acid in water. Air was bubbled through the liquid and forced out a tube that lead to the bag containing the test individuals. Air was then vented out a tube into a fume hood. Fumigation was constant for 12 days.

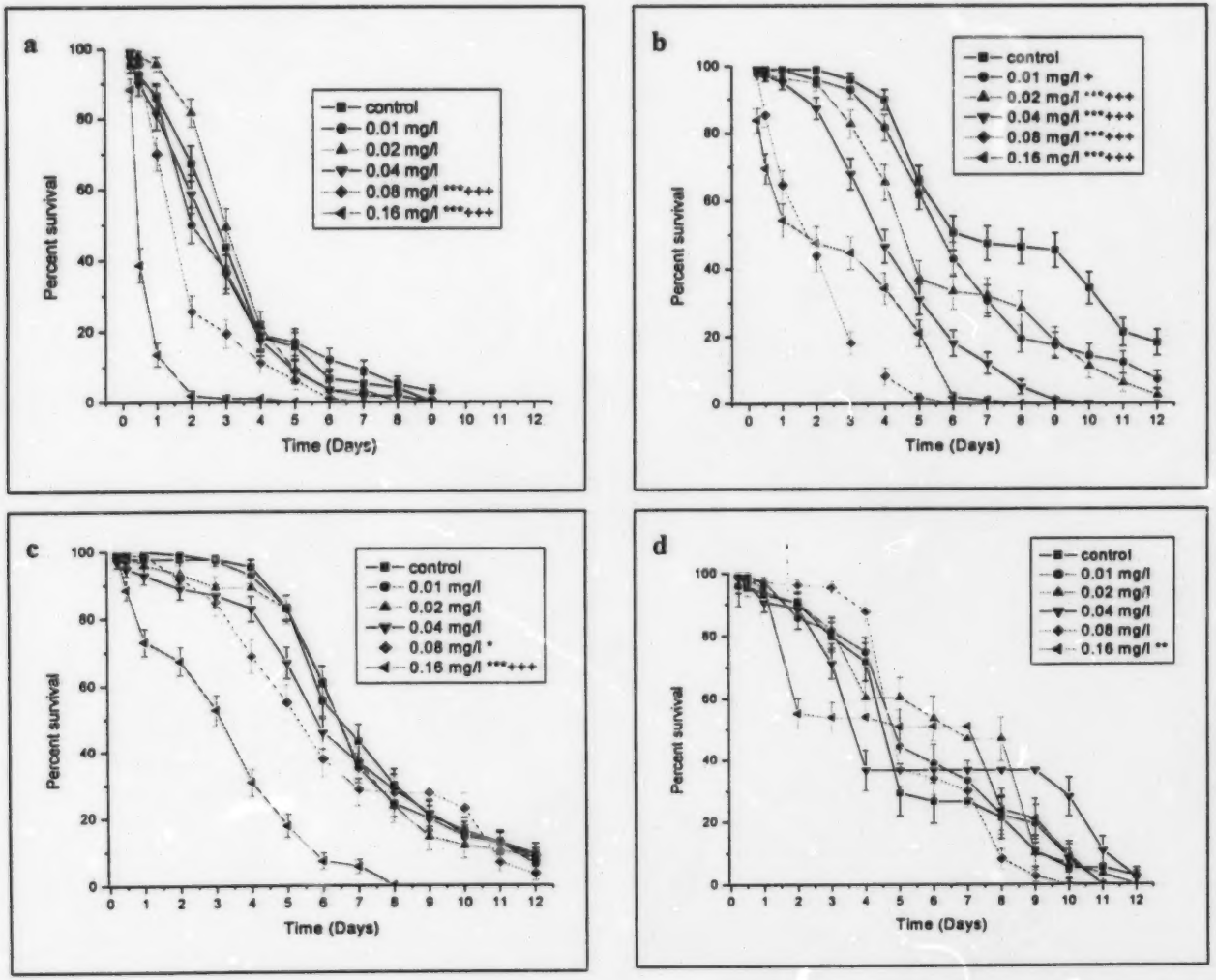

Figure 2. *Varroa* mite survival ±SD at 35°C (a), 25°C (b), 15°C (c), and 5°C (d) over the duration of the experiment. N for each point is approximately 90 mites. Significant differences between a treatment and the control are denoted as *P<0.05, **P<0.01, ***P<0.001 according to the Wilcoxon test and +P<0.05, ++P<0.01, and +++P<0.001 according to the Log-Rank test of the LIFETEST procedure.

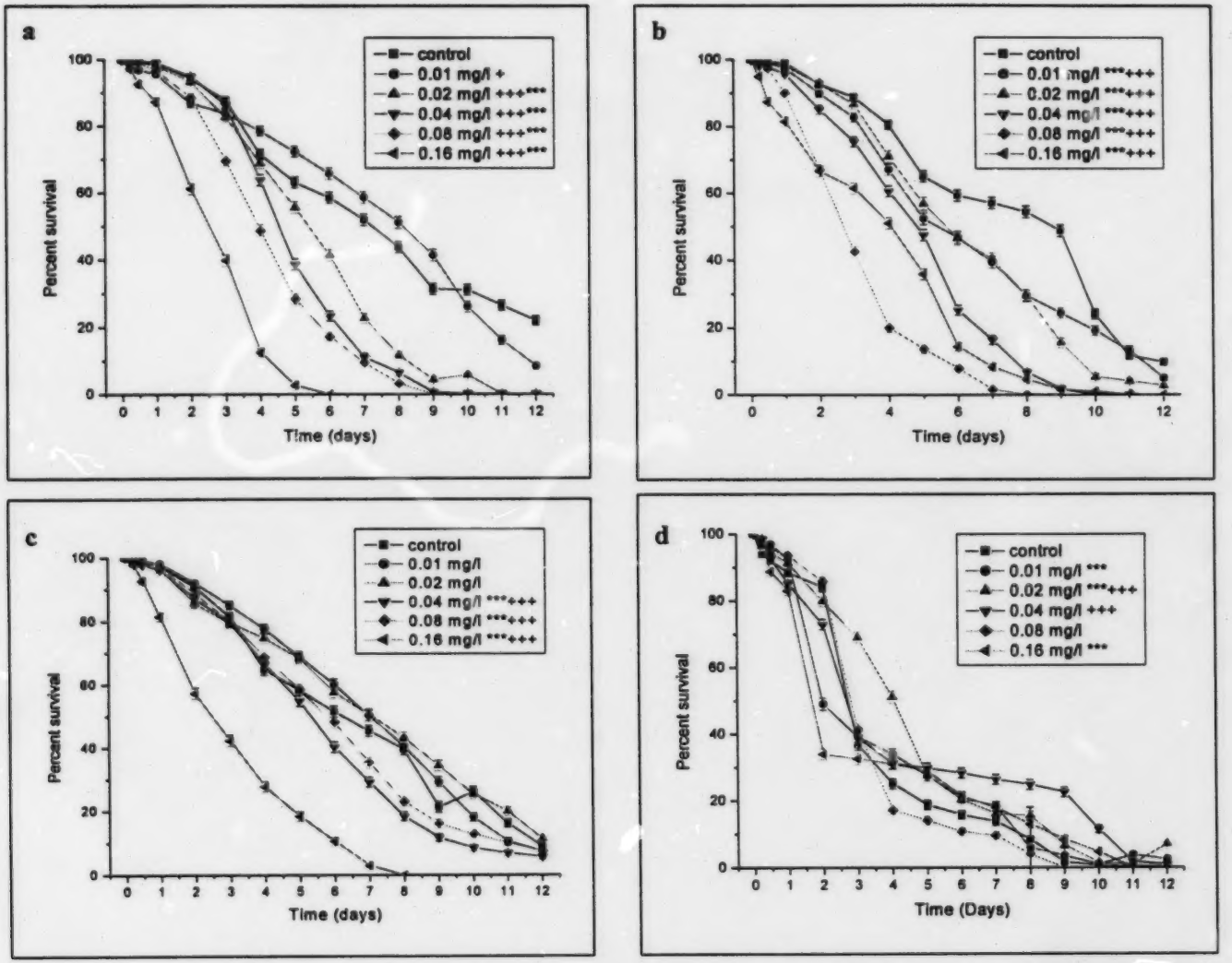

Figure 3. Worker bee survival ±SD at 35°C (a), 25°C (b), 15°C (c), and 5°C (d) over the duration of the experiment. N for each point is approximately 900 bees. Significant differences between a treatment and the control are denoted as *P<0.05, **P<0.01, ***P<0.001 according to the Wilcoxon test and +P<0.05, ++P<0.01, and +++P<0.001 according to the Log-Rank test of the LIFETEST procedure.

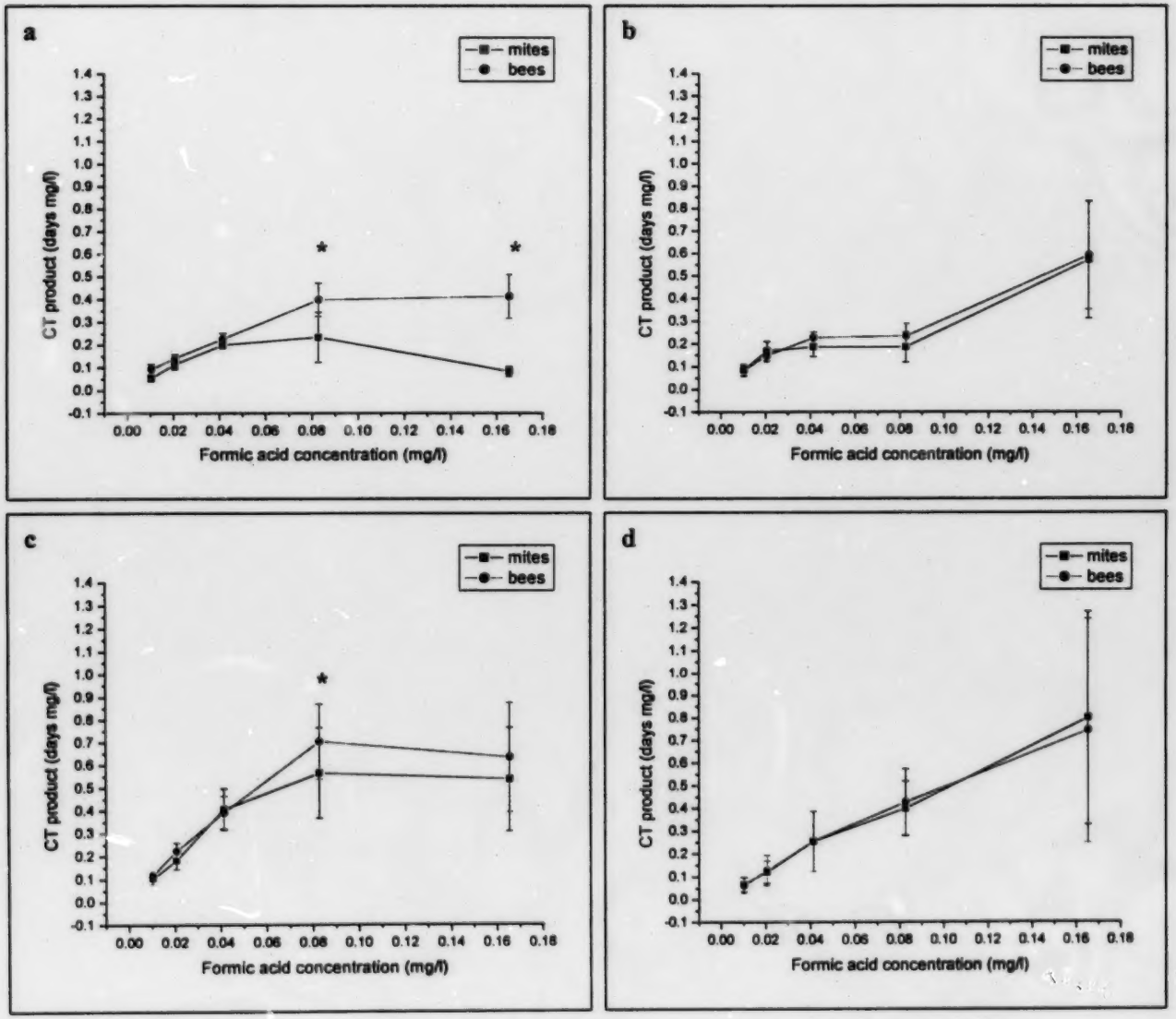

Figure 4. CT products for bees and mites at 35°C (a), 25°C (b), 15°C (c), and 5°C (d). Points are means ± SD for 3 replicates. Values are corrected for mortality in the control using Abbott's formula. Significant differences between the CT product of bees and mites are denoted by a * and all P values are <0.01.

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Apiculture and Social Insects

Underwood and Currie: Indoor winter fumigation with formic acid

Robert W. Currie

Department of Entomology

University of Manitoba

Winnipeg, MB R3T 2N2

(204)474-6022 (phone)

(204)474-7628 (fax)

currier@ms.umanitoba.ca

Indoor winter fumigation of *Apis mellifera* L. (Hymenoptera: Apidae) colonies infested with *Varroa destructor* Anderson and Trueman (Acari: Varroidae) is a practical control alternative in northern climates

ROBYN M. UNDERWOOD AND ROBERT W. CURRIE, DEPARTMENT OF ENTOMOLOGY, UNIVERSITY OF MANITOBA, WINNIPEG, MB R3T 2N2 CANADA

Abstract

Formic acid treatment for the control of the ectoparasitic mite, *Varroa destructor* Anderson and Trueman infesting honey bee, *Apis mellifera* L., colonies is usually an in-hive spring treatment. This study examined the use of formic acid in a practical experiment on wintered colonies kept indoors in darkness at 5°C from 24 November 1999 to 24 March 2000. Colonies were placed in small rooms that were fumigated for 48 hours on 22-24 January 2000. Queen and worker bee and parasitic mite mortality were monitored throughout the winter. This study revealed that formic acid fumigation of indoor-wintered honey bees is feasible and effective. The highest dose caused the highest mite mortality without increasing bee mortality. *Nosema* levels were significantly lower in the high dose treatment then in the control after the experiment. Tracheal mite levels did not change significantly, although a treatment effect may have been obscured by methodology. Queen loss was seen at unacceptable levels. Repeated fumigation periods may increase the efficacy of this treatment method and should be tested in future studies. The cause and prevention of queen loss must be revealed for this method to be generally accepted.

Key words: formic acid, *Varroa destructor*, overwintering, *Apis mellifera*, fumigation

Introduction

As early as 1918, beekeepers in the Canadian prairies relied on the importation of U. S. honey bees, *Apis mellifera* L., to replenish stocks each spring because of the harsh winters experienced there (citation for Beekeeping for the Beginner, MB Dept of Ag). In the late 1970's and early 1980's, prairie beekeepers became interested in overwintering their colonies, but imports still accounted for about 30% of Canada's bees (Dixon 1999). With the arrival of the ectoparasitic mite *Varroa destructor* Anderson and Trueman in the U. S. in 1987 (DeJong 1990) closure of the U.S.-Canadian border was necessary to prevent its spread into Canada (Johnson 1988). In the absence of available packaged bees, prairie beekeepers were forced to overwinter more colonies. Despite the border closure, *Varroa destructor* arrived in Canada in 1990 and is now well established there (Anonymous 1991). The border remains closed today and beekeepers have continued to overwinter their bees.

Honey bee colonies can be overwintered indoors or outdoors. Outdoor wintering involves placing hives in a sheltered location and wrapping them with insulation that serves to slow down temperature changes, conserve heat, and decrease moisture within the hives (Gruska 1998). To winter bees indoors, hives are moved into a building that is maintained at approximately 5°C in darkness with proper air circulation and ventilation (Gruska 1998). Under these controlled environmental conditions, honey bee colonies survive very well even in areas with the harshest of winters.

During the early winter months in northern regions, little or no honey bee brood is present in the hive (Winston 1987). At this time of year, *Varroa destructor*, which reproduces in the cells with developing honey bees, also halts its reproduction and must rely on adult honey bees for food (DeJong 1990). Therefore, winter may be an ideal time to treat *Varroa*-infested

colonies because most of the mites will be exposed to treatment. In the prairie region of Canada where reinfestation of colonies is limited, a single treatment during this yearly gap in mite reproduction may provide sufficient control of *Varroa* mites for the entire year.

One treatment method that may be suited for use indoors in winter is formic acid fumigation. Currently, formic acid is used outdoors as an in-hive fumigant. This study is an assessment of the feasibility of using formic acid fumigation to control *Varroa* mites in an overwintering building. A number of formic acid doses were tested on honey bee colonies infested with *Varroa destructor* being overwintered indoors. The effects of different concentrations of formic acid on the pests *Varroa destructor*, *Nosema apis* and *Acarapis woodi* were monitored. Worker bee, queen bee, and overall colony survival were also measured.

Materials and Methods

Timeline of events. Eighty-four honey bee, *A. mellifera*, colonies were obtained from southern Manitoba beekeepers in early November 1999 and brought to the University of Manitoba apiary in Winnipeg, Manitoba, Canada. On November 24, 1999, colonies were assessed to determine initial pest infestation levels and colony characteristics before being moved into the overwintering building on the University of Manitoba campus. On January 12, 2000, hives were moved into experimental rooms within the overwintering building and set up for the experiment. Experimental rooms were fumigated with formic acid for 48 hours from January 22-24, 2000. All hives remained in the building until March 23, 2000 when they were moved outside to the University of Manitoba apiary. Final pest infestation levels and colony components were measured on March 24, 2000.

Pre-treatment sampling. Each colony was contained in one full-depth Langstroth hive body containing 9 or 10 frames. On November 24, 1999, all 84 hives were sampled to assess

worker cluster size, *Varroa destructor*, *Nosema apis*, and *Acarapis woodi* infestation levels, and hive weight. Cluster size was ranked and given a score from one to three using the following criteria: a score of one was a cluster covering three or fewer frames, a score of two was a cluster covering four to six frames, and a score of three was a cluster covering seven or more frames. A sample of 200-300 adult worker bees was collected from each colony to estimate parasite and disease loads. *V. destructor* infestation level on adult workers was estimated by the alcohol wash method as follows. Workers were placed in a box (26.5 by 15.0 by 8.5 cm) with a screen floor (hardware cloth, 6 squares per inch) raised 1 cm from the bottom of the box. The bees and box were placed in a larger closed plastic container (30.0 by 9.0 by 8.5 cm, Rubbermaid®, Wooster, OH) containing 1500 ml of 70% ethanol. The entire container was shaken on a mechanical shaker (Lab-line Instruments, Inc., Melrose Park, IL) for approximately 10 minutes at 1500 rpm. The number of mites that dropped through the screen was divided by the number of bees washed to get an estimate of *Varroa* mite abundance in each colony.

After each sample was washed, 100 bees were taken from each sample for an assessment of *N. apis* and *A. woodi* levels. *N. apis* levels were assessed according to the methods of Shimanuki and Cantwell (1978). *A. woodi* levels were assessed according to the methods of Delfinado-Baker (1984). After the pre-treatment samples were taken, the hives were moved into the overwintering building at the University of Manitoba and maintained there under constant darkness at 5°C.

Hive setup. On January 12, 2000, 21 hives were moved into each of four small rooms (3.0 by 1.7 by 2.7 m; Figure 1) within the overwintering building. Hives were grouped into blocks based on initial *V. destructor* infestation level and hive weight. Hives within each group were randomly assigned to each treatment.

Once the hives were in place in the experimental rooms, hives 1-5 (Figure 1) were fitted with temperature probes (HOBO® data logger, Onset Computer Corporation, Bourne, MA). These colonies were given a disturbance rank of zero while all others were given a rank of one. In all rooms but the control room, hives 1-3 were also fitted with 4.3 mm inner diameter polyethylene air-sampling tubes (Anderson Barrows Metals Corporation, Palmdale, CA). HOBO®s and air-sampling tubes were attached directly to the comb with a thin wire and located at the edge and center of the honey bee cluster. Each air-sampling tube ran from the point of measurement to a site outside the room. Each tube was sealed on the end located outside the room with a rubber sleeve stopper (5.2-6.7 mm plug diameter, Fisher Scientific Limited, Ottawa, ON, 03-215-5). Thus, air samples could be collected during fumigation without entering the room.

A piece of white poster board (36 by 56 cm) was placed on the bottom board of each hive to allow fallen *V. destructor* and bees to be collected throughout the experiment. Additional dead bees were collected outside of the hive entrance in a dead bee trap (aluminum, three-sided box, 47 by 19 by 8 cm; Figure 1g). All bees and mites falling onto the white board or into the aluminum pan were collected from all 84 hives at six points throughout the winter. The first collection consisted of a two-day, pre-treatment period from January 19-21. The second collection was for a five-day period (during and immediately after fumigation) from January 21-27. Thereafter, collections were made every 14 days until March 21, 2000. All workers, queens and *V. destructor* that were found on boards or in the dead bee traps from each sample were counted to calculate an average daily mortality rate for each collection period.

Room temperature and air flow. Each experimental room simulated the air flow conditions of a commercial wintering building (Gruska 1998), but was modified to allow formic

acid fumigation (Figure 1). An air mixing chamber (Figure 2) was designed to blow air into the room at a rate of 106 liters per second. Air was taken from the outside (through a chimney, Figure 2b) or recirculated from the room itself (Figure 2c). Baffles were opened or closed manually to direct airflow as necessary. Air was continuously distributed throughout the room through a plastic ventilation tube (6.7 cm radius) that ran the length of the room and had 10 evenly spaced 5 cm diameter holes (Figure 1d). Air velocity from each hole was approximately 5 m per second. Additional fans sucked air out of the room to provide a minimum air exchange at a rate of 0.24 liters per second per hive and a maximum of 6.43 liters per second per hive in steps from one to four (Gruska 1998) (Figure 1a-c). The walls of each room were covered with thin plastic (0.5 mil Polyester/3 mil Polyolefin Plastomer Coextrusion, Winpak Ltd., Winnipeg, MB) to prevent air flow between the rooms. In addition, the door of each room was sealed for the 48 hours of treatment and the subsequent 48 hours (January 22-26, 2000). The plastic was stapled to the walls and across the doors. All seams were covered with duct tape. A baseboard heater (Figure 1f) and thermostat maintained each room at approximately 5°C.

Temperature probes and air-sampling tubes were used to measure room air in the center of the room at heights of 0.1, 0.5, and 1.0 m. They were suspended from the ceiling and anchored to the floor with cotton string. An additional length of string ran from the device to the back wall.

Fumigation. In order to distribute the formic acid throughout the rooms, a fan and pan system was set up in the air mixing chamber (Figure 2). A pan (25.0 by 25.0 by 5.5 cm; Figure 2g) of liquid was placed in front of a fan (10 cm diameter window fan, Holmes Products Corp., Milford, MA; Figure 2f). A 10 liter plastic bag (Reliance Products Ltd., Winnipeg, MB; Figure 2a) was connected to the pan by a tube (0.4 cm inner diameter, Anderson Barrows Metals

Corporation, Palmdale, CA). The bag acted as a reservoir to replace liquid that evaporated from the pan. Flow of liquid between the bag and the pan was regulated by a float (Air-King Limited, Brampton, ON). During fumigation, each chimney baffle was closed and each room baffle was open. The vents for the step three ventilation fans (Figure 1c) were sealed with thin plastic and duct tape to prevent air flow between the rooms.

Based upon previous testing in empty rooms, various humidifier plates and dilutions of formic acid in the pan and reservoir (Underwood and Currie, unpublished data) were used to manipulate formic acid concentrations in the air of the experimental rooms. Four application methods were designed to expose bee colonies in different rooms to different concentrations of formic acid while the fourth room was a control (Table 1). The pan for the control room started with 1 liter of water, a humidifier sponge (30 by 19 by 5 cm, Air-King Limited, Brampton, ON) and a bag with 8 liters of water. The low dose treatment started with a pan containing 1 liter of 65% formic acid, an identical humidifier sponge and a bag with 8 liters of 65% formic acid. The medium dose treatment started with a pan containing 1 liter of 55% formic acid, three T-shaped humidifier plates (11.5 by 16.7 by 0.2 cm, Dundas-Jafine Industries Ltd., 64-3119-0, Weston, ON) and a bag with 8 liters of 65% formic acid. The high dose treatment started with a pan containing 1 liter of 85% formic acid, three identical humidifier plates and a bag with 8 liters of 85% formic acid.

Formic Acid Sampling. Throughout the bioassay, air samples were taken for formic acid concentration measurement using an air monitoring system (National Dräger, Pittsburgh, PA). Immediately prior to obtaining each sample, the entire volume of air was withdrawn from the sealed tube and discarded using a disposable syringe. An air sample was then collected by attaching a needle to a Dräger tube (Tube number 6722101) with a piece of rubber tubing. The

needle pierced the stopper and allowed air to be sucked into and through the Dräger tube and into the gas detector pump (Accuro 9022003). The volumes of air were drawn through the pump according to the manufacturer's instructions (National Dräger, Pittsburgh, PA). The Dräger tube allowed an immediate measurement of acid concentration measured in parts per million (ppm).

Formic acid concentration was measured only in the three "fumigated" rooms. Measurements were taken from air samples taken from the center of fumigated rooms at heights of 0.1 m, 0.5 m and 1.5 m and from hives at the center and perimeter of the honey bee cluster in three hives in each room, one at each height in the center column of the stacked hives in row 1 (Hives 1-3; Figure 1). Air samples were taken at hours 2, 6, and 12 at all locations. At hours 24, 48, and 72, measurements were taken at the edge and center of the cluster in hives 1-3, but only at the 0.5 m height in the center of each room.

Temperature was measured continuously using HOBO® data loggers (Onset Computer Corporation, Bourne, MA) at the same sites used for formic acid measurements. Additional temperature measurements were taken at the center and perimeter of the cluster in hives 4 and 5 (Figure 1) to monitor hives different distances from the heater. Temperature was also monitored from similar sites in hives 1-5 (Figure 1) in the control room.

Post-treatment. After 24 hours of fumigation, the liquid was removed from the pans and the reservoir bags were emptied. The chimney baffles were opened to allow fresh air into the rooms. Additional ventilation fans were turned on to increase the air exchange to approximately 0.71 liters per second per hive (step two ventilation rate). The rooms were ventilated in this way until January 26, 2000 when the door seals were removed and the fans were turned back to step one. For the remainder of the winter, the fans were controlled by thermostats and activated in series when room temperatures rose above 5°C (Gruska 1998).

Throughout the rest of the experiment, colonies were fed a 67% sucrose solution *ad libitum* using a hive-entrance feeder made of a 454 gram plastic honey container. Small holes were pierced through the container, which was turned upside down. The sugar syrup solution was captured in the lip of the lid where it was maintained by gravity and surface tension at a constant level.

On March 23, 2000, all hives were removed from the overwintering building and placed outside in the University of Manitoba apiary. Each hive was sampled the following day to assess worker cluster size, *V. destructor*, *N. apis*, and *A. woodi* infestation levels, and hive weight as described above. An additional cluster size rank of zero was used to describe a dead colony. An additional measurement of the final *V. destructor* population was taken by placing one strip of 10.25 % Fluvalinate-tau (Apistan®, Sandoz Agro Canada, Inc., Guelph, ON) near the center of the cluster in each hive. A sticky white board (Phero Tech Inc., Delta, BC) covered by a screen (hardware cloth, 6 squares per inch) on the bottom board of each hive collected fallen mites for 48 hours (March 25-26, 2000). All mites that fell to the floor of the hive during this period were counted.

Data Analysis. Analysis of Variance (ANOVA) was used for all analyses using PROC GLM in the SAS program (SAS Institute 1999). A split-plot design was used for cluster size, *Varroa destructor* infestation level, and hive weight data collected at the beginning and end of the experiment and for worker bee and *Varroa* mite fall throughout the winter. These tests used treatment and block as main-plot factors and time as the subplot factor. *V. destructor* infestation level data were analyzed after arcsine transformation ($\arcsin(\sqrt{x/103})$). Division by 103 was necessary because levels up to 103 mites per 100 bees were recorded. *Nosema apis* and *A. woodi* levels were analyzed after log transformation ($\log_{10}(x+1)$). When significant differences were

found ($P<0.05$), a Student-Newman-Keuls' (SNK) multiple range test was used to compare differences between means. For *N. apis* data, contrasts were used to compare each treatment to the control.

Comparisons of formic acid concentration levels and temperatures based on height and position of measurement were analyzed using a split-split-plot design. Treatment was the main plot factor, location (height) and position (room vs. edge vs. center) were the subplot factors, and time was the sub-subplot factor. When significant differences ($P<0.05$) were found, a Student-Newman-Keuls' (SNK) multiple range test was used to compare differences between means. The effect of disturbance on colony survival was analyzed by ANOVA with treatment as the main plot factor and disturbance rank as the block. *V. destructor* fall counts during Apistan® treatment were analyzed using a randomized complete block design. Correlations between colony survival and colony weight and disturbance rank were analyzed using PROC CORR in the SAS program.

Results

Formic acid concentration. The total amount of liquid evaporated into each room is shown on table 1. Formic acid measurements in hives and in the room at various heights indicated that the acid was well distributed throughout the room (ANOVA, $F=0.09$, $df=2$, $P=0.919$). Within the room, formic acid measurements did not differ with height from a range of 0.1 m to 1.5 m. Similarly, the bottom, middle and top hives received similar amounts of formic acid.

During the 48 hours of fumigation, the acid concentration in the room was significantly higher than that in the hives (ANOVA, SNK, $F=18.64$, $df=2$, $P=0.001$, SNK; Figure 3). However, the acid concentration did not differ significantly within the hives between the edge

and the center of the worker cluster (SNK). Within the room, the concentration of acid remained fairly stable during fumigation and quickly dropped off after the formic acid was removed from the pan (Figure 3). Within the hives, formic acid concentration increased slightly with time, but was lower than the acid concentrations in the rooms until 24 hours after fumigation ended. Once the acid was removed from the air mixing chamber, the concentration of acid in the hives remained relatively stable for at least 24 hours, whereas the acid levels in the rooms dropped dramatically. In the high and low dose treatments, the measurements taken 24 hours after the acid was removed from the pans showed that there was more acid in the hives than in the room.

***V. destructor* mortality.** The average mite fall per day during fumigation was significantly higher than the mite fall in samples taken before and after fumigation (ANOVA, $F=43.32$, $df=5$, $P<0.001$, SNK; Figure 4). During fumigation, average daily mite fall was significantly affected by treatment (ANOVA, $F=9.89$, $df=3$, $P<0.001$). A multiple range test (SNK) revealed that the high dose treatment caused significantly more mortality than the other three, the medium and low dose treatments were not significantly different from each other and the control and low dose treatments were not significantly different from each other.

Worker bee mortality. Honey bee worker mortality was not significantly affected by formic acid fumigation. Mortality was similar for all treatments (ANOVA, $F=1.04$, $df=3$, $P=0.382$). However, there was a significant difference in average daily bee fall over time (ANOVA, $F=5.55$, $df=5$, $P<0.001$). This difference was not associated with fumigation. The samples taken immediately before and immediately after fumigation were significantly different from samples taken during fumigation and four and eight weeks after fumigation (SNK).

Queen bee mortality. In the high dose treatment, five out of 21 (24%) queens fell during the fumigation sampling period and an additional two (9.5%) were lost after fumigation. The control and medium dose treatments also lost one queen (4.8%) each during the experiment.

***Varroa destructor* abundance.** In the fall, there was no significant difference in mite level between treatments (ANOVA, $F=1.03$, $df=3$, $P=0.387$). In the spring, however, mite levels differed significantly (ANOVA, $F=7.86$, $df=3$, $P<0.001$). The high dose treatment had significantly fewer mites per bee than the other three treatments (SNK; Figure 5).

Cluster size. The average cluster size decreased significantly over the course of the experiment (ANOVA, $F=8.68$, $df=1$, $P=0.005$). However, this change was not due to a treatment*time interaction (ANOVA, $F=2.15$, $df=3$, $P=0.103$). An ANOVA of cluster size after the experiment shows that treatment did not affect the final cluster size ($F=0.96$, $df=3$, $P=0.417$). Thus, even the size of the clusters in the control room decreased.

Colony survival. Treatment did not significantly affect colony survival (ANOVA $F=1.20$, $df=3$, $P=0.319$). Overall colony survival measured immediately after hives were removed from the building was 75%. The control and high dose rooms had the lowest colony survival at 67% each. The low and medium doses had the highest colony survival, with 81% and 86% survival respectively.

Hive weight decreased significantly between measurements taken before and after the experiment ($F=865.08$, $df=1$, $P<0.001$), but this was not related to treatment ($F=0.26$, $df=3$, $P=0.8508$). The differences in colony weight were not correlated with colony survival ($P=0.1306$) and the differences in colony weight were negatively correlated with disturbance ($P=0.0387$).

Placement of air-sampling tubes and temperature probes in hives midwinter disturbed the colonies. This disturbance had a negative impact on colony survival. Colonies in hives 1-5 (Figure 1) in each room had a significantly higher probability of dying than those that were not fitted with tubes and probes (ANOVA, $F=14.60$, $df=1$, $P<0.001$).

***Nosema apis* level.** There was a change in *N. apis* levels from the beginning to the end of the experiment ($F=2.96$, $df=3$, $P=0.04$). At the end of the experiment, levels of *N. apis* were significantly lower in the high dose treatment than in the control ($F=5.07$, $df=1$, $P=0.03$). In the control, low and medium dose rooms, *N. apis* levels drastically increased over the course of the experiment (Figure 6). In contrast, in the high dose treatment, levels decreased slightly (Figure 6).

***Acarapis woodi* level.** There was no significant effect of treatment on tracheal mite levels measured as the number of bees with infested tracheae per 100 bees sampled ($F=0.88$, $df=3$, $P=0.46$). Numerically, levels increased in the control over the course of the experiment, whereas, in all other treatments, levels decreased. However, this decrease was not statistically significant.

Discussion

Treatment of *Varroa*-infested honey bee colonies indoors in winter by formic acid fumigation is feasible. This method uses less formic acid and less manpower than traditional methods of formic acid treatment. Indoor fumigation did not affect bee mortality but was effective at increasing *V. destructor* mortality and decreasing *N. apis* levels at some doses. Fumigation did not impact overall colony survival or *A. woodi* levels. Colonies exposed to high concentrations of formic acid experienced some queen loss. These results suggest that large-

scale fumigation with formic acid can be effective, but more work is required before this treatment can be used commercially.

Formic acid concentration. The amount of formic acid used during treatment in the overwintering building was considerably lower than the amount needed for treatment of individual colonies outdoors; less than 50 ml per hive indoors as compared to 250 ml per hive outdoors (e.g. Stoya et al. 1986, Calderone and Nasr 1999). In addition, labor costs for this form of fumigation are undoubtedly less than that required for the use of formic acid outdoors on individual colonies.

The even distribution of formic acid in the experimental rooms indicates that it will be possible for beekeepers to keep their hives stacked at least three high during fumigation. Because the ventilation system used in this experiment was designed to simulate a commercial overwintering building (Gruska 1998), the results are applicable to a large scale overwintering situation.

The formic acid concentration in the room was higher than that in the hives themselves. Therefore, future researchers must continue to measure acid concentration in these areas separately. Elucidating the cause of the difference may be important for standardization of this method. In addition, the fact that the acid concentration in the air in the hives remained stable for at least 24 hours after treatment ceased needs to be taken into account. Absorption of acid into stored honey and winter feed may be important.

***V. destructor* mortality.** Treatment with formic acid indoors in winter may be useful as a *V. destructor* control alternative. During fumigation, mite mortality increased with dose of formic acid, as expected. However, only the high dose treatment brought the average *Varroa* mite infestation level to below 2%, the point at which economic losses occur (D. Dixon, personal

communication). The low and medium doses killed more mites than the control treatment, but the overall effect on *Varroa* mite population levels was not adequate. The duration of fumigation is suspected to be the cause of the low efficacy in low and medium dose treatments. Long-term fumigation at these doses may be effective at controlling mites and may also decrease the frequency of queen mortality.

During this study, mites were collected on "non-sticky" white boards. Therefore, any mites that fell to the bottom board, but were still alive, may have climbed back onto the bees. This was done to ensure that only those mites actually killed by the treatment were counted. The treatment itself would have been more effective if all of the fallen mites had become stuck to the white board or had fallen through a screen bottom board.

Worker bee mortality. Fumigation with formic acid in winter did not increase worker bee mortality, regardless of dose. Thus, colony survival was not significantly affected by treatment. Numerically, the colonies treated with low and medium doses were the most likely to survive until spring. If this is not due to *V. destructor* control, perhaps it is due to some other factor, such as a decrease in the *A. woodi* infestation, that was not observed.

As expected, hives decreased in weight over the course of the experiment. This change was similar among rooms, indicating that formic acid fumigation did not increase the intake of food. Disturbance in the middle of the winter had a negative impact on colony survival. If the hive is opened and the colony is disturbed at an inopportune time, the probability that the colony will collapse before spring is increased. Future studies should avoid disturbance whenever possible.

Queen bees. The high level of queen loss observed after the high dose treatment is unacceptable. Queen loss has been seen in previous studies (Fries 1989, VonPosern 1988), but

the cause of death has not been determined. Future studies are needed to elucidate the cause of queen death and to develop a strategy for queen mortality prevention.

***N. apis* and *A. woodi* level changes.** This is the first study that documents effective control of *Nosema apis* with formic acid. The high dose treatment prevented winter build-up of *N. apis* infestation. While the mode of action of formic acid on *N. apis* is unknown, we suspect that treatment may render spores inviable.

In this study, we did not see an impact of formic acid treatment on tracheal mite levels. This is most likely because our methods did not allow us to detect the changes. We dissected dead bees and counted the number of bees with infested tracheae. Thus, we were not able to discern live from dead mites. Examination of live bees would have been necessary to distinguish between bees infested with live mites from those with mites killed by formic acid treatment, but remaining in the tracheae. Future researchers must examine live bees in order to determine the effectiveness of winter fumigation in controlling tracheal mites.

Summary. The results of this study show that indoor winter fumigation with formic acid is feasible. Formic acid is easily distributed throughout the room and doses high enough to reduce *Varroa* mites to an acceptable level were attained. Future studies that focus on perfecting the dose, timing, and/or number of treatments required to bring mite levels below economic threshold levels will enable the marketing of this mite control method. Reducing or eliminating queen mortality during treatment will also be critical for the adoption of this treatment practice.

Formic acid fumigation of indoor-wintered colonies will save chemical and labor costs. All hives can be treated simultaneously, thus decreasing the probability that the applicator will be injured. Many beekeepers in the prairies currently winter their bees indoors and would only need to modify their mixing chambers in order to fumigate with formic acid. Because formic

acid is also useful in controlling tracheal mites and wax moths (Hoppe et al. 1989, Feldlaufer et al. 1997), its use may have a greater impact than that mentioned here.

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References Cited

Anonymous. 1991. *Varroa* mites detected in Manitoba swarm traps. American Bee Journal 131:745-746.

Calderone, N. W., and M. E. Nasr. 1999. Evaluation of formic acid formulation for the fall control of *Varroa jacobsoni* (Acari: Varroidae) in colonies of the honey bee *Apis mellifera* (Hymenoptera: Apidae) in a temperate climate. Journal of Economic Entomology 92:526-533.

DeJong, D. 1990. Mites: *Varroa* and other parasites of brood, pp. 200-218. *In* R. A. Morse and R. Nowogrodski [eds.], Honey bee pests, predators and diseases. Cornell University Press, Ithaca, NY.

Delfinado-Baker, M. 1984. *Acarapis woodi* in the United States. American Bee Journal 124:805-806.

Dixon, D. 1999. The Canadian mite experience. Proceeding of Apimondia '99, 107-108.

Feldlaufer, M. F., J. S. Pettis, J. P. Kochansky, and H. Shimanuki. 1997. A gel formulation of formic acid for the control of parasitic mites of honey bees. American Bee Journal 137:661-663.

Fries, I. 1989. Short-interval treatments with formic acid for control of *Varroa jacobsoni* in honey bee (*Apis mellifera*) colonies in cold climates. Swedish Journal of Agricultural Research 19:213-216.

Gruska, J. 1998 Beekeeping in Western Canada. Alberta Agriculture, Food and Rural Development, Edmonton, Alberta.

Hoppe, H., W. Ritter, and E. W. C. Stephen. 1989. The control of parasitic bee mites: *Varroa jacobsoni*, *Acarapis woodi* and *Tropilaelaps clareae* with formic acid. American Bee Journal 129:739-742.

Liu, T. P. 1991. Formic acid and bee mites. American Bee Journal 131:311-312.

SAS Institute. 1999. SAS/STAT user's guide. SAS Institute, Cary, NC.

Shimanuki, H. and G. Cantwell. 1978. Diagnosis of honey bee diseases, parasites, and pests. ARS-NE-87.

Stoya, W., G. Wachendörfer, I. Kary, P. Siebentritt, and E. Kaiser. 1986. Formic acid as a therapy against Varroatosis and its absorption into honey. Deutsche Lebensmittel-Rundschau 82:217-221.

VonPosern, H. 1988. Stopping *Varroa*'s victory march part II. American Bee Journal 128:425-428.

Winston, M. 1987. The biology of the honey bee. Harvard University Press, Cambridge, MA.

Table 1. Formic acid evaporation during the 48 hours of fumigation. The final amount of acid in the pan and in the reservoir minus the initial amount in the pan and in the reservoir gives the total amount of acid evaporated during fumigation in each room.

Treatment	Initial pan amt. (ml/%FA)	Initial bag amt. (ml/%FA)	Total evaporated (ml)	ml per hive
Control	1000/0	8000/0	150*	7.1*
Low Dose	1000/65	8000/65	650	31
Med. Dose	1000/55	8000/65	300	14.3
High Dose	1000/85	8000/85	750	35.7

*Estimated as some water froze in the pan.

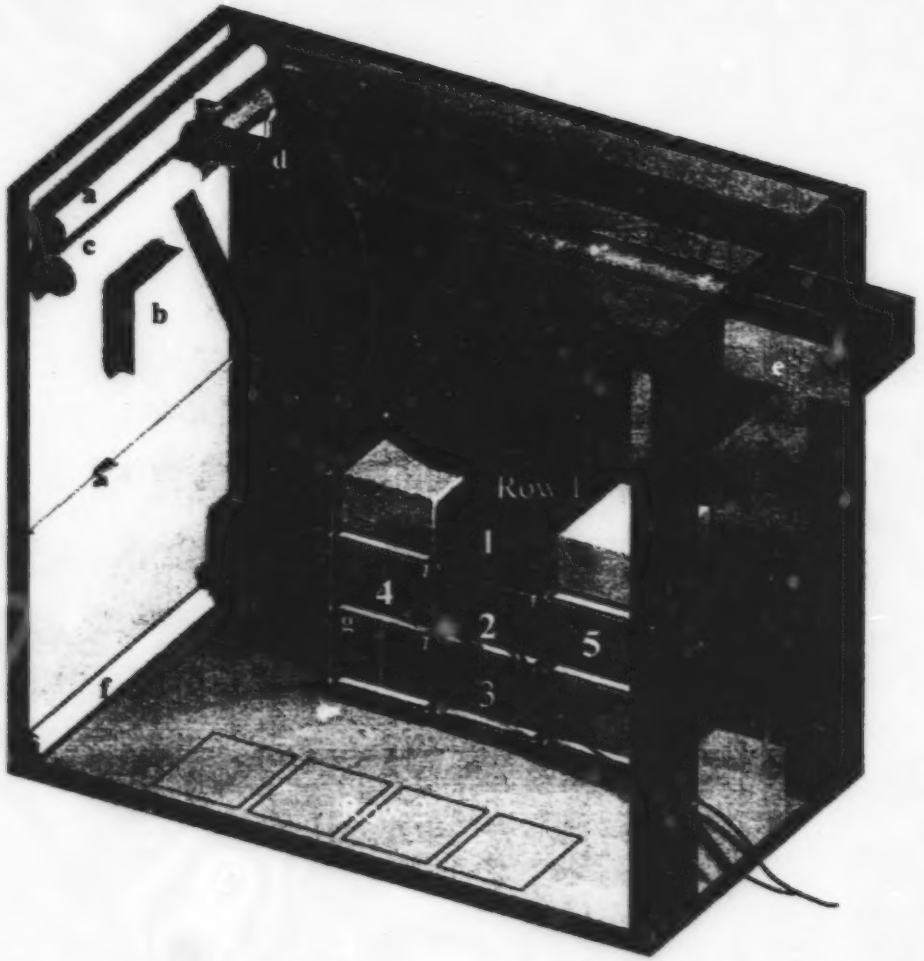

Figure 1. Setup of an experimental room. Missing from the diagram: twelve hives in row 2, tubes coming from hives 2 and 3, and tubes used to measure the formic acid concentration in the center of the room. a) ventilation tube for steps one and two, b) ventilation fan for step three (covered by a light trap (Gruska 1998)), c) ventilation tube for step four, d) tube for formic acid distribution, e) air mixing chamber (see Figure 2), f) baseboard heater, g) dead bee trap. Hives 1 through 5 are discussed in the methods text.

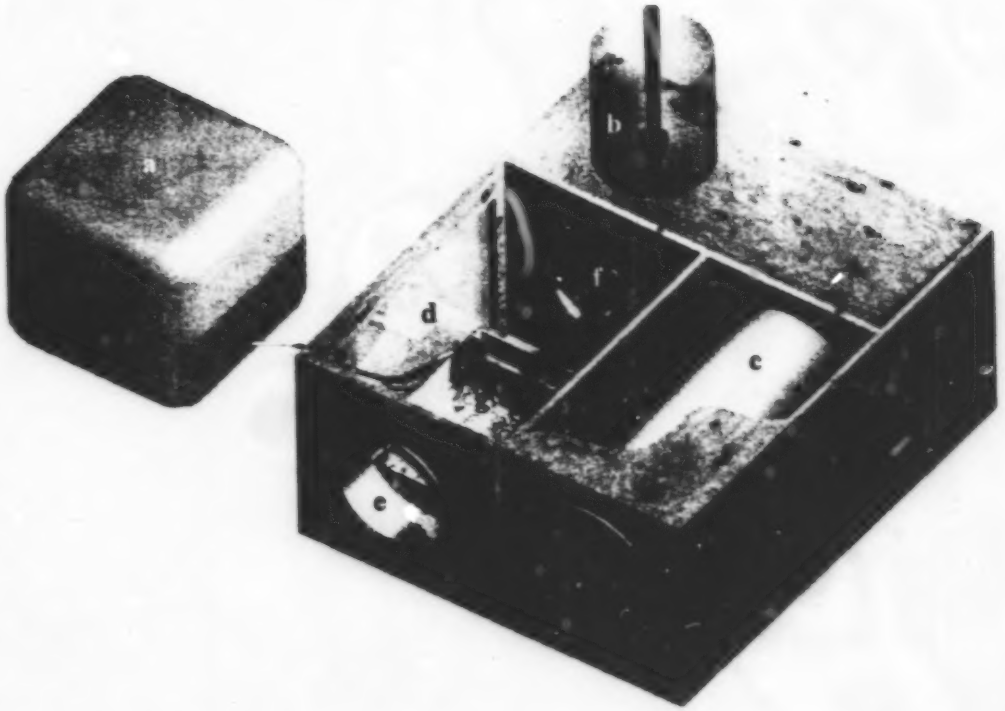

Figure 2. Air mixing chamber. a) reservoir bag, b) chimney to allow fresh air into the room, c) pipe to allow room air to be recirculated, d) window for addition and removal of formic acid, e) connects mixing chamber with distribution tube in the room (Figure 1d), f) fan blowing constantly throughout the winter, g) pan containing liquid and a float. Arrows indicate the direction of air flow.

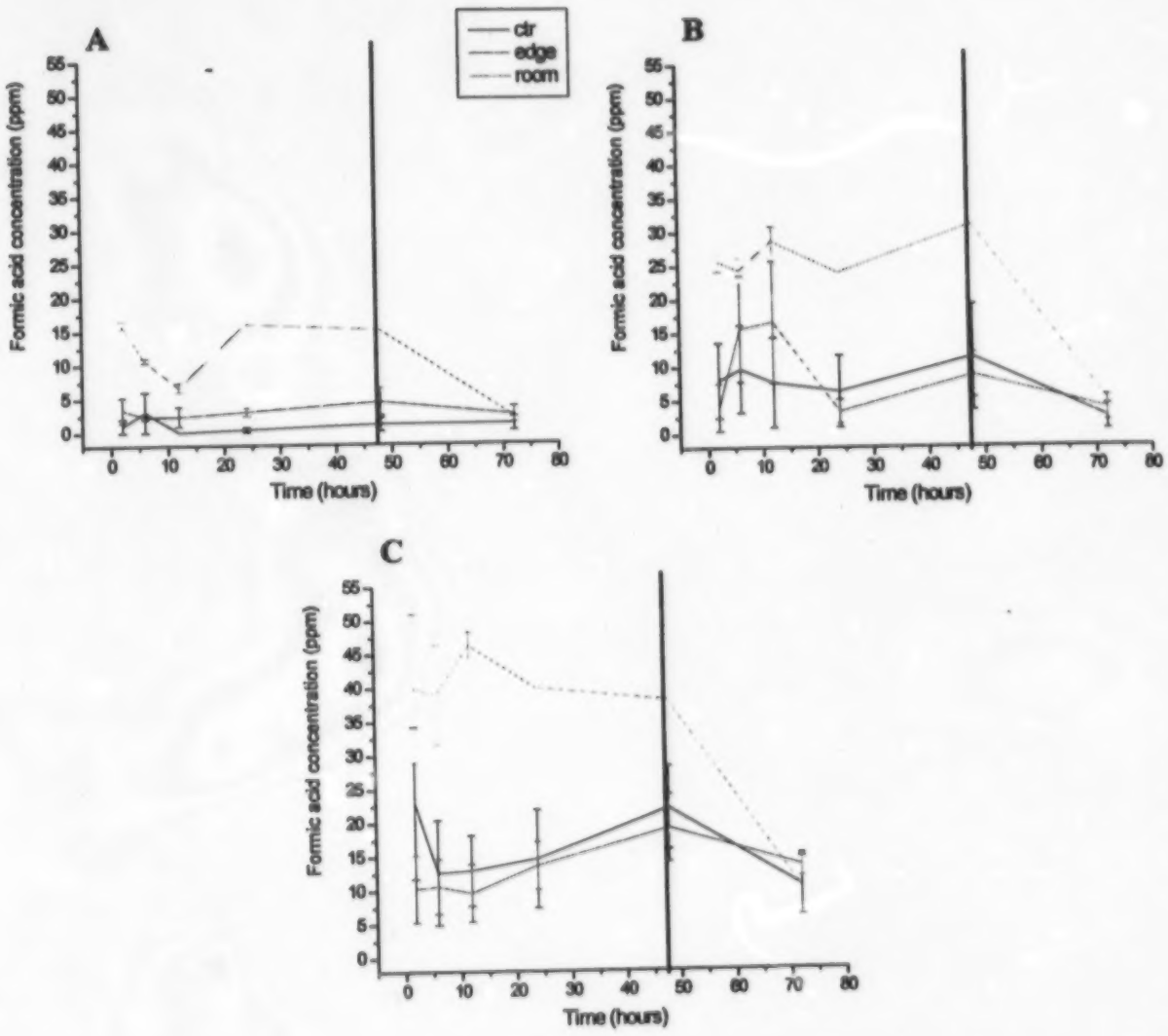

Figure 3. Formic acid concentration measurements in air (parts per million) in experimental rooms over the 48 hours of fumigation and beyond. Each line represents an average of three measurement sites (except the room measurements after hour 12 that represent only one measurement). The vertical line represents the time when the acid was removed from the pans. A) Measurements in the low dose treatment. B) Measurements in the medium dose treatment. C) Measurements in the high dose treatment. For each treatment, the concentration in the room was significantly higher than that in the hives.

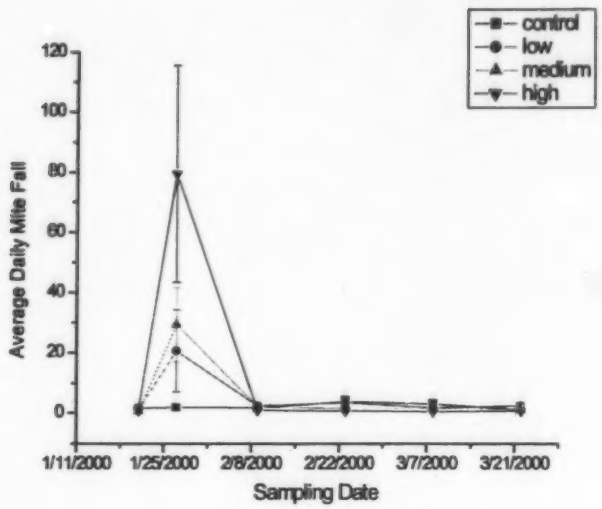

Figure 4. Average daily *Varroa* mite fall from collections from white boards and dead bee traps. Each line represents the mite fall (an average of 21 hives±SD) from a given treatment. Mite fall was counted for each sampling period and divided by the number of days of collecting to get a daily average.

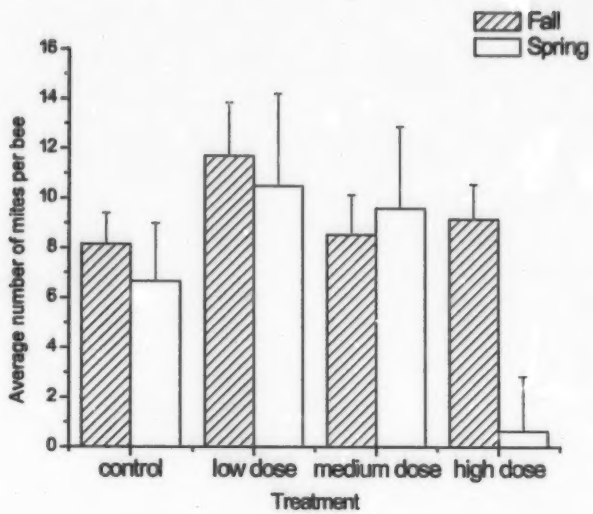

Figure 5. Average *Varroa* infestation level ±SD (of 21 hives per treatment) in each room before (fall) and after (spring) the experiment.

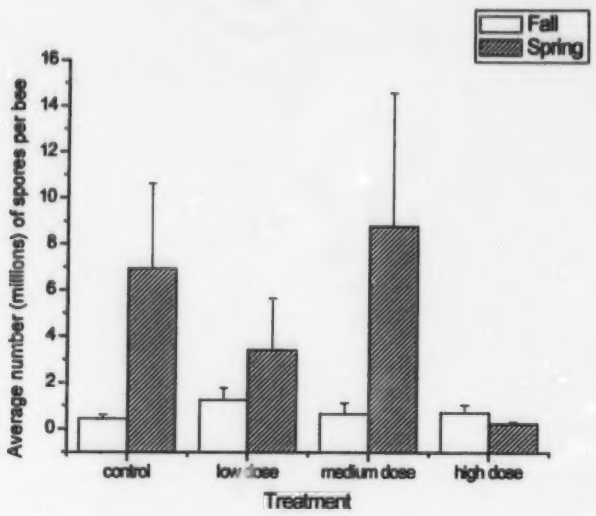

Figure 6. Mean number of *Nosema apis* spores per bee ±SD for colonies that survived until the March 24, 2000 sampling date. Fall samples were taken on November 24, 1999 and spring samples were taken on March 24, 2000.